Studies on the Olfactory Bulbs of the Albino Rat—in Two Parts

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From The Wistar Institute of Anatomy and Biology

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STUDIES ON THE OLFACTORY BULBS OF THE ALBINO RAT—IN TWO PARTS

I. EFFECT OF A DEFECTIVE DIET AND OF EXERCISE

II. NUMBER OF CELLS IN BULB

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FOUR PLATES

PART I. EXPERIMENTS TO DETERMINE THE EFFECT OF A DEFECTIVE DIET AND OF EXERCISE UPON THE WEIGHT OF THE OLFACTORY BULBS

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[1] Thesis presented to the Faculty of the Graduate School of University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

I. INTRODUCTION

The various members of the mammalian series show considerable variation in the relative development of all parts of the central nervous system, but probably no part of the encephalon shows so great a degree of variability as does the rhinencephalon. Of this portion of the brain, the olfactory bulbs are, without doubt, the most variable in size. Thus we have the very large bulbs of the opossum and the ant-eater; the almost rudimentary bulbs of the ape and of man; extreme reduction of these organs in the Cetacea, with their complete disappearance in the dolphin. Not only do we find variation in size of the olfactory bulbs among the different orders of mammals, but we find that there is a considerable degree of variability within each order and even among the members of the same species.

This variation in size and weight of the olfactory bulbs within a species is well illustrated by observations upon the rats in the colony of The Wistar Institute. The domesticated albino rats exhibit a considerable range in the development of this part of the brain. But while we find an appreciable difference in the bulb size of rats of different litters even under like environmental conditions, the individuals of a given litter usually show a more uniform development of the olfactory system. Some wild Norway rats examined at The Wistar Institute a few years ago had olfactory bulbs heavier in proportion to total brain weight than the bulbs of the albino. In the course of the present study, observations made upon some thirty wild Norway rats caught at different places in Philadelphia suggested that this difference between the two strains is not a constant one, for,

while the olfactory bulbs of these animals were much heavier than those of the albinos, the ratio between bulb weight and brain weight in this series was about the same in the two forms.

Inequality in the size of the two bulbs in the same individual appears not infrequently in the albino and when it occurs it will often be found in several, and occasionally in all, members of the same litter. For this reason, in selecting material for these experiments, we discarded all litters in which cases of asymmetry were observed among the initial controls.

Observations made from time to time by Dr. Donaldson, indicated that rats born in the early summer differ from winter-born rats in the relative size of the olfactory bulbs; also that there might be a difference between rats reared on a restricted diet, such as is frequently used in colonies, and those fed on the table-scrap diet adopted for the Wistar colony. Moreover, cases had appeared in which the bulbs of sick rats were apparently smaller than those of healthy individuals.

All these facts suggested that there might be factors in the living conditions of the rats which would account for the variability of this portion of the nervous system, the growth of the bulbs being retarded or arrested in rats reared under unfavorable conditions, such as the intense heat of the summer, or a monotonous diet, or in those suffering from the various infections which may attack the rats from time to time.

It was, therefore, with the hope of throwing some light upon the question of the effect of environmental conditions upon the olfactory bulb of the growing albino rat, that, at the suggestion of Dr. Donaldson, the present experiments were undertaken. The problem resolved itself into two questions—Can the growth of the olfactory bulbs of the stock albino be modified (1) by underfeeding or (2) by exercise?

The writer wishes here to express her deep gratitude to Dr. Donaldson for his unfailing helpfulness and encouragement, and her appreciation to Dr. Stotsenburg and Dr. Heuser, and to the other members of The Wistar Institute who did much to aid in the course of the experiments which have extended over the past two years.

II. DEFECTIVE DIET EXPERIMENTS

1. Previous experiments on the effect of starvation upon the central nervous system of the rat

There have been several previous studies upon the effect of underfeeding and of starvation upon the central nervous system of the albino rat. In 1904, Hatai reported experiments on 'partial starvation' for twenty-one days. He fed large quantities of starch with some fat but no proteids of any kind. He obtained a deficiency in body weight of 27 per cent in females and 32 per cent in males. Taking the values from the initial controls for a standard, the brains of the test rats showed, at the end of twenty-one days, a deficiency of 2.8 per cent for the females and 5.8 per cent for the males. Thus the treatment produced not only an arrest of brain growth but a loss in absolute brain weight. This experiment was followed by a series in which the animals, after a defective diet (Oswego starch), were returned to a normal diet. Here Hatai ('07) found that the effect of twenty-one days of partial starvation was eventually compensated for, so far as brain weight was concerned, but the central nervous system had suffered some change in its chemical composition. The following year ('08), Hatai published the results of further experiments, this time in quantitative underfeeding with an adequate ration, in which he concludes that growth in the stunted rats is just as normal as in the controls; i.e., all parts are proportionately stunted.

In 1911, Donaldson published an account of the effect of underfeeding, with a quantitatively deficient, but adequate ration, on the percentage of water, on the ether-alcohol extractives and on medullation in the central nervous system of albino rats, showing a slight diminution of percentage of water, slight increase in percentage of ether-alcohol extractives, and no notable difference in medullation.

Jackson ('15) found, in young rats maintained at a constant weight on a diet of bread and milk, that the relation between body weight and brain weight remained unchanged. The brain

ceased to grow simultaneously with the body, while the cord increased somewhat in weight during underfeeding.

Although there are, at present, no data by which it is possible to make a definite comparison between the effect of a defective diet and that of an adequate, but quantitatively insufficient diet, it is important to bear in mind in each case the method by which growth has been retarded or arrested.

Two series of experiments upon the effect of underfeeding were undertaken—one upon rats which had been reared to the time of weaning (at three or at four weeks of age) by well-fed mothers, Series A; the other, upon rats reared to the time of weaning by underfed mothers, which meant rats underfed practically from birth, Series B.

Observations were also made upon a few sick animals, Series C.

2. Series A. A_1, rats underfed from time of weaning at eighteen to twenty days, and A_2, at thirty to thirty-two days

a. Method. As has been previously stated, while there is a considerable range of variation between litters in the matter of the relative size of the olfactory bulbs, yet within a given litter the size is fairly uniform. For this reason, so far as possible, control and test animals were taken from the same litter. This, of course, made it necessary to select fairly large litters in order to have several animals for initial and final controls, and also for experiment. The litters were always taken from healthy stock animals.

For the first few individuals experimented upon, no initial controls were examined, but the results of these experiments made the advisability of such controls apparent and subsequently each litter was weighed and divided into three groups; so far as possible, equivalent in sex, weight, and bodily condition. All these rats were ear marked and a card filed for the data upon each animal.

The first or initial control animals were at once etherized, weighed, measured, eviscerated, and the brains removed. One olfactory bulb was cut off from each brain, in the following man-

ner. The brain was placed, ventral side down, on the dissecting board. Then with a thin, sharp scalpel held in a position perpendicular to the plane of the board and at right angles to the plane of the median longitudinal fissure, the bulb was severed just below the anterior limit of the cerebrum.[2] The bulb, with the remainder of the brain was then placed in a covered weighing bottle and the weight of both the entire brain and of the severed bulb ascertained.

The final controls were weighed and placed under the normal living conditions of the colony: i.e., housed, in long wooden cages with wire fronts, thick shaving-covered floors, and paper nests, and given plenty of fresh water with a carefully supervised scrap diet. The test rats were weighed and placed in adjoining cages under exactly the same conditions as the final controls, save for the diet. The food given the test rats consisted of an unlimited amount of whole corn, usually fed on the cob, save in case of very young animals, or those weak from a long period of underfeeding. In such cases, the corn was shelled as the animals were not able to remove a sufficient amount for themselves.

Both control and test animals were weighed from time to time and the weights recorded. Note was also made of any irregularities, such as a temporary change in diet, etc.

At maturity, a certain number of test and of control animals were mated in order to find out whether underfeeding affected the fertility of albino rats.

In the case of relatively small litters in which the members were usually well grown and in good physical condition when weaned—and especially if weaning was delayed until the rats were four weeks old—it was possible to keep the test animal on a corn diet for a month or more with practically no difficulty.

[2] Small bulbs tend to differ characteristically in shape from large ones. On section it is seen that the cap of gray substance extends somewhat further caudad on the ventral surface of the small bulb than it does in the case of the large bulb. The weight of gray substance thus lost in the case of the small bulb is a very small fraction of the total weight of the bulb but a much larger fraction of the gray cap. Care must, therefore, be taken to include this portion when the number of cells of the gray substance is to be determined.

With the rats weaned at three weeks or in case of small rats from very large litters, there was a good deal of trouble in keeping the animals on the corn diet for so long a time, and of course the difficulty increased as the period of underfeeding was prolonged.

At first an attempt was made to keep animals from several litters in one cage with the result that after a short time, the less well grown rats were killed and eaten by the stronger individuals. Then the plan was adopted of having members of only one litter in a cage. This worked successfully up to the time when the animals began to weaken. Then the males frequently killed and ate the females. So finally, for prolonged experiments, it was found safer to place only animals of the same sex, approximate weight and physical condition together, but even this precaution was not always sufficient.

In most cases, for the first few weeks, there was a very slow gain in weight or the weight was just maintained. But in every case when an animal began to lose or became very feeble, a dose of condensed milk was fed. One or two doses were usually sufficient to restore the animal to equilibrium and there was not infrequently a sudden temporary gain in weight, doubtless due to increased appetite and the consequent gorging of the alimentary tract with corn.

In a few cases where the underfeeding had gone on for several months, it became necessary to administer small doses of condensed milk more frequently—in two cases, practically every day—in order to keep the animals from losing weight.

At the end of the experiment, both test and final control animals were killed, weighed, measured, eviscerated, and brains and bulbs weighed as in the case of the initial controls. One bulb with a part of the cerebrum was preserved for histological study. A record was kept of any signs of disease or other abnormality. The weighing was done in closed bottles and all weights of brain and of olfactory bulbs were made to 0.1 mgm., but recorded here in milligrams only.

b. Results. General morphological and physiological modifications. A summary of the data from observations upon 108

individuals of Series A is given in tables 1 to 8. The complete tables with the records for each individual rat of this, as well as of the other series, are deposited at The Wistar Institute. Of the two litters weaned at eighteen and twenty days, only three individuals survived to be killed; the others died in the cages and the brains were not weighed. The records for the three rats just named have been included in tables 3 and 7, and their controls, with the corresponding controls. The size and body weight of rats weaned at the end of the third week and placed on a corn diet indicated clearly that under like conditions, rats weaned at three weeks are considerably more sensitive to adverse conditions than are those weaned at four weeks.

For every individual of Series A_1 and A_2 (tables 1 to 8), the stunting effect of the corn diet was apparent almost from the first. During the early weeks of underfeeding the test rats appeared rather more lively than the controls. Later this activity decreased, the gait became unsteady, and the animals appeared stupid. They were often unable to find the dish of condensed milk by themselves, whereas control rats would go to it immediately. This suggests that the underfed animals lacked an acute sense of smell and perhaps did not see clearly.

In every one of the test animals of which there are complete records, the general bodily growth was arrested by a diet of corn. This agrees with the observations of Osborne and Mendel ('13). These rats remained like young animals in appearance as well as in size. The earlier weaning took place and the corn diet was begun, the more complete the stunting.

The skeleton became modified and somewhat distorted owing to imperfect calcification. The growth of the long bones was not quite so completely arrested as that of the rest of the skeleton. The skull, sternum, and sometimes the ribs, became like parchment. In two cases the pressure of the heart upon the sternum had formed a sort of pocket out of that structure, which appeared like a tumor on the ventral side of the rat. The vertebral column became somewhat bowed, giving to the rat a 'humped' appearance and making it necessary to stretch the animals when measuring body length. One to four months of

underfeeding, following the first month under normal conditions, left the rats but slightly longer (4 to 10 mm.) than the initial controls measured at thirty days. The average increase in weight was in about the same proportion. Compare tables 2 to 8, for body weight and body length.

All the rats showed extreme emaciation but this condition was largely masked by the condition of the coats. The hair remained short and soft, with a fluffiness which gave even to mature rats the appearance of plump young animals. Such emaciation was, of course, accompanied by great muscular weakness. Rats kept for long periods on the defective diet became unable to remove corn from the cobs. They walked with a tottering gait and moved about but little.

The cyanosed condition of these animals was clearly indicated by the blue color of all exposed parts of the body—nose, ears, feet and tail. In protracted cases of underfeeding, a chronic palpitation of the heart developed which increased in violence as time went on. As a result of this, the whole body shook constantly.

All animals kept on corn up to maturity failed to breed or to show any sexual instinct whatever.

Effect on brain and olfactory bulbs (compare tables 1 to 8). In Series A, both A_1 and A_2 show a slight increase in brain weight during the period of underfeeding. Under normal conditions, as the rat grows, the brain becomes relatively lighter in proportion to body weight. In the underfed rats the brain forms practically the same proportion of the total body weight as in the initial control rats (agreeing with Jackson's results ('15)), which of course indicates in the cases where growth has taken place that the brain has not been as much arrested in its development as has the rest of the body.

After four to eight weeks of underfeeding, the rats of Series A_1 and A_2 had olfactory bulbs which, taken together, formed about the same proportion of the total brain weights as did the bulbs of the initial controls of the same series, showing that the relation of these parts of the brains had not been changed during the experiment. But normally the olfactory bulbs grow faster

during this period than the rest of the brain so that at eight weeks, for example, the bulbs should form a considerably greater percentage of the total brain weight than at thirty days. The average absolute weight of the bulbs of the test animals was equal to but 70 to 81 per cent of the average weight of the bulbs in the control animals of the same series. It is therefore evident that the retarding effect of underfeeding has been greater upon the olfactory bulbs than upon the other parts of the brain, which had 85 to 90 per cent of the weight of the brains in the control series.

If the relative weight of the bulbs in Series A_1 and A_2 is determined for the test group as contrasted with the final control group, we obtain the following relations:

TABLE 1

	GROUP	AGE	PERCENTAGE WEIGHT OF OLFACTORY BULBS
		days	
Table 3.....	Test rats, defective diet	60	3.52
Table 4.....	Final controls	60	3.99
Table 5.....	Test rats, defective diet	79	3.39
Table 6.....	Final controls	79	4.16
Table 7.....	Test rats, defective diet	118	3.83
Table 8.....	Final controls	128	4.30

This arrangement of the results shows clearly that in each of the three sets, grouped according to age, the olfactory bulbs of the underfed rats are significantly lighter in proportional weight than those of the controls. We may, therefore, conclude that the relative weight of the olfactory bulbs is reduced by the form of defective feeding employed in this experiment.

The details are given in tables 2 to 8, which follow.

3. Series B. Rats on deficient diet from birth

a. Method. Since it was evident that the earlier the animals were weaned, the greater the stunting effect of a qualitatively inadequate diet, it occurred to the writer that it would be interesting to try underfeeding from birth, by underfeeding the

TABLE 2. SERIES A

Initial control animals

In all of the tables the averages are weighted for the number of animals in each entry

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS: PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
14 males	30	44.1	118	1.439	0.051	3.52	3.01–3.97
11 females	30	43.5	116	1.409	0.050	3.53	2.41–4.19
Averages for males and females		43.8	117	1.426	0.050	3.53	2.41–4.19

TABLE 3. SERIES A

Test animals

Stock albinos kept on corn diet for twenty-nine to forty-two days after weaning at three to four weeks

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS: PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
15 males	60	55.5	126	1.505	0.052	3.45	2.38–4.53
11 females	60	53.9	126	1.502	0.054	3.62	2.68–4.21
Averages for males and females		54.8	126	1.504	0.053	3.52	2.38–4.53

TABLE 4. SERIES A

Final control animals

Stock albinos kept on normal diet for twenty-nine to forty-two days after weaning at four weeks

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS: PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
19 males	61	127.4	167	1.668	0.066	3.95	2.76–4.62
7 females	60	95.1	154	1.606	0.065	4.04	3.66–4.53
Averages for males and females		118.8	164	1.651	0.066	3.99	2.76–4.62
Summary $\frac{\text{Test}}{\text{Control}}$			77	91	81		

TABLE 5. SERIES A

Test animals

Stock albinos kept on corn diet for forty-nine days after weaning at four weeks

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS: PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
2 males	80	47.8	126	1.502	0.055	3.63	3.31–3.93
1 female	78	37.8	116	1.458	0.042	2.89	
Averages for males and females			124	1.487	0.050	3.39	2.89–3.63

TABLE 6. SERIES A

Final control animals

Stock albinos kept on normal diet for forty-nine days after weaning at four weeks

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS: PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
2 males	80	155.0	178	1.727	0.068	3.93	3.72–4.14
1 female	78	151.2	183	1.703	0.079	4.63	
Averages for males and females		153.8	180	1.719	0.072	4.16	3.72–4.63
Summary $\frac{\text{Test}}{\text{Control}}$			69%	78%	70%		

TABLE 7. SERIES A

Test animals

Stock albinos kept on corn diet for fifty-nine days or more, after weaning at four weeks. (One rat weaned at eighteen days)

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS: PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
7 males	120	47.1	118	1.463	0.057	3.86	3.47–4.19
6 females	115	54.3	126	1.594	0.060	3.79	3.55–4.29
Averages for males and females		50.5	122	1.524	0.058	3.83	3.47–4.29

TABLE 8. SERIES A

Final control animals

Stock albinos kept on normal diet for fifty-nine days or more, after weaning at four weeks

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS: PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
8 males	121	201.4	199	1.806	0.078	4.29	3.81–4.73
4 females	136[1]	157.0	181	1.706	0.074	4.32	4.03–4.62
Averages for males and females		186.7	193	1.772	0.076	4.30	3.81–4.73
Summary $\frac{\text{Test}}{\text{Control}}$			63%	85%	77%		

[1] This higher average age for the female controls is due to the fact that one female was kept for breeding purposes until two hundred and thirteen days old. As all her measurements were practically identical with those of another female of same litter, one hundred and fifteen days old, the record was included in the table.

mothers which were bearing or nursing the young to be tested. Consequently nine pregnant females were selected. A few were put on a corn diet several days before the birth of the young, but most of them began the corn feeding on the day of the birth of the litter. The young rats were weaned at three weeks and fed exclusively on corn.

It is intended to carry out this experiment more extensively at some future time but enough animals were tested to give significant results. It was found very difficult to raise such litters, for two reasons. In the first place, after the young reached an age to leave the nest, the mother was very apt to kill the entire litter. This, apparently, was not because of hunger, for in all but two cases in which the young rats were partially eaten, the animals were mutilated only to the extent of a bite through the cerebellum, and sometimes through the front of the throat. It has been suggested that the increasing demands of the young, coupled with an inadequate milk supply, may have been the cause of this unnatural behavior of the mothers.

But the chief reason for the difficulty in raising these rats was their lack of vitality. Although very active and playful, these animals were extremely frail little creatures, so weak that the slightest disturbance was likely to prove fatal. For example, an unusually active and promising test rat of fifty-three days, was carried from the colony to the laboratory for examination. As he appeared much excited, the carrier cage was set aside for an hour. The rat was heard running about for a time but at the end of the hour was found dead. The body weight of this rat was that of an animal fifteen days old and the brain weight was scarcely more. Young rats might appear lively and in every way normal in a late afternoon and be found dead in the cage next morning, for no reason to be discovered even after careful examination. Of nine such litters only two survived to the time of weaning, and these were kept with much difficulty.

A litter of 'runts' was also included in this series. This was a litter of rats, all of which failed to grow normally, presumably because the mother had an insufficient supply of milk. They appeared in every way like the rats which had been stunted by underfeeding the mothers.

b. Results. The general results of underfeeding in Series B were essentially the same in character as in Series A but they were considerably more marked (tables 9, 10 and 11). The body length and general appearance of seventy-seven day rats, underfed from birth, were practically the same as in normal three-weeks-old rats, save for the extreme cyanosed condition.

From a comparison of Series A_1, A_2, and B, it becomes evident that it is easier to retard the growth of an eighteen day rat than of a rat thirty days old, and still easier to stop the growth of a rat at about the size of an eighteen day individual if the underfeeding is begun at birth. Moreover, it is obviously far more difficult to rear these animals underfed from birth than rats which have been allowed to get a good start of thirty days under favorable conditions and are therefore much more resistant to the deleterious effects of partial starvation.

Effect on brain and olfactory bulbs. Series B shows brains actually lighter in weight at twenty-four to fifty-three days of

age than normal brains of seventeen days (The Rat, table 74). Rats seventy-seven days old had brains weighing practically the same as those of normal female rats of forty-two days.

Bulbs of rats twenty-four to fifty-three days old, actually weighed only 70 per cent as much as those of normal rats of thirty days (table 2) and only 52 per cent as much as bulbs of normal rats eight weeks old (table 4).

Rats eleven weeks old (table 11) gave bulbs of the same absolute weight as those of control rats of thirty days (table 2). In both cases the olfactory bulbs formed a smaller per cent of the total brain weight than appeared among the controls of like age in Series A, as the following arrangement of the data shows:

TABLE 9

	GROUP	AGE	PERCENTAGE WEIGHT OF OLFACTORY BULBS
		days	
Table 10.....	Test rats, defective diet-Series B.	24–53	3.14
Table 2.....	Control.	30	3.53
Table 11.....	Test rats, defective diet-Series B	77	3.48
Table 4.....	Control	60	3.99
Table 6.....	Control	79	4.16

The details for these series are given in tables 10 and 11 which follow.

4. Series C. Sick rats

In the course of the experiments a number of sick rats came under observation. Eleven of these were examined to determine whether the brain, and especially the olfactory bulbs, showed any effects of the diseased condition. Three of these rats were the sole survivors from a group of twelve attacked by a serious bowel trouble which killed the other nine occupants of the cages. At the time of the onset of the illness, the rats were about eighty days old. After about ten days, these three seemed to recover and were kept until they were about a hundred and thirty-five

TABLE 10. SERIES B

Test animals

Stock albinos underfed from birth. Under two months old

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
6 males	24–38	20.1	86	1.114	0.036	3.19	3.06–3.38
4 females	24–53	15.6	79	1.062	0.033	3.08	2.11–3.82
Averages for males and females		18.1	83	1.091	0.034	3.14	

TABLE 11. SERIES B 1.

Test animals

Stock albinos underfed from birth. Over two months old

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
3 females	77	31.2	102	1.437	0.050	3.48	3.15–3.93

days old when they were killed and examined. The other eight sick rats of this Series C were individuals showing a considerable infection of the lungs, and one of these (No. 20) had, in addition, a large abscess of the liver.

All of these rats were examined in the same way as those of Series A.

a. Results. In the group of sick animals, those with the intestinal infection had, at one hundred and thirty-four days, bulbs which averaged 0.050 gram or 3.02 per cent of the total brain weight (table 12, group 1) while a set of normal individuals of practically the same age gave an average of 0.073 gram or 4.32 per cent of the total brain weight (see table 20, group of females). These results seem especially interesting because here the adverse conditions appeared only after the rats were well grown—eighty days old—and lasted only about ten days.

The remaining two groups of sick rats all had infected lungs and were very old when killed. The two males had bulbs

averaging 0.037 gram, or 2.08 per cent of the total brain weight (table 12, group 2); while for the four females, the bulbs averaged 0.033 gram, 1.89 per cent of the total brain weight (table 12, group 3). For these last two groups there are no data of normal individuals for comparison but the percentage for the bulbs is strikingly low. Some unpublished data in Dr. Donaldson's hands show, however, that while the relative weight of the olfactory bulbs tends to increase up to about one hundred and fifty days of age, in older rats there is a tendency to decrease so that some of this decrease observed in the old sick rats (groups 2 and 3) may be due to normal age changes. But the remarkably small proportional weight of the bulbs here examined is probably due chiefly to the effect of disease.

In this connection may be mentioned two young rats of litter PR (group 2), killed at seventy days. Each had infected lungs. These rats came from parents with infected lungs and had lived since birth in a dark damp cage. One had very small unequal bulbs which were not weighed. The other had bulbs weighing only 0.019 gram or 1.30 per cent of the entire brain weight. This pair of bulbs were the smallest observed in the whole series of experiments. It seems quite evident that the bulbs are abnormal and quite probable that this abnormality is due to disease.

5. Summary and conclusions. Defective diet experiments

1. General bodily growth in the albino rat is arrested by an exclusive ration of corn which constitutes a defective diet (Osborne and Mendel).
   a. The skeleton is poorly calcified and somewhat distorted.
   b. The muscular system is greatly reduced.
   c. The coat has the appearance of that of a young animal.
2. Functional disturbances follow the arrested development.
   a. There is increasing muscular weakness.
   b. An increasing palpitation of the heart.
   c. The animals appear cyanosed.

TABLE 12. SERIES C

Sick animals

Females

Group 1. Three albino rats from a lot of twelve controls for revolving cage experiment. At about eighty days all contracted a severe bowel trouble from which these three recovered.

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS PER CENT OF BRAIN WEIGHT
	days	*gm.*	*mm.*	*gm.*	*gm.*	
Z_1	135	128.5	165	1.607	0.033	2.03
Y_{22}	135	150.5	180	1.534	0.034	2.24
X_3	134	157.9	182	1.803	0.082	4.55
Average females		145.6	176	1.648	0.050	3.02

Males

Group 2. Two, old, with infected lungs.

PR_1	70	88	148	1.489	0.019	1.30
PR_6	70	90	155	1.538	unequal	
No. 24	old	213	202	1.759	0.050	2.81
No. 69	365	214	195	1.791	0.024	1.35
Average Numbers 24 and 69		213.5	198	1.775	0.037	2.08

Females

Group 3. Infected lungs, old age, and in case of No. 20, bad abscesses on liver.

No. 20	340	143.0	173	1.673	0.027	1.63
No. 21	340	146.1	187	1.751	0.032	1.79
No. 71	370	186.0	186	1.687	0.051	3.01
No. 72	240	218.3	199	1.794	0.021	1.16
Average females		173.4	186	1.726	0.033	1.89

d. The sense organs become dulled after prolonged defective feeding—the animals respond but slowly to stimulations of sound, or light or smell.

e. Defectively fed animals fail to breed.

3. The effect of defective feeding on the brain and olfactory bulbs is less than upon the rest of the body, but is, nevertheless, very marked. The olfactory bulbs are stunted and to a con-

siderably greater degree than is the entire brain. When defective feeding is begun in rats about thirty days of age, the bulbs of rats thus experimentally stunted form about the same percentage of the total brain weight as do the bulbs of rats of the same litters killed at the beginning of the experiment. Whereas, under normal conditions, the bulbs of older rats (up to one hundred and fifty days) are considerably heavier in proportion than those of the young animals. With prolonged defective feeding the proportional weight of the bulbs tends to become slightly greater.

4. Sick animals, especially those with lung infection, show a marked diminution in the relative weight of the olfactory bulbs, accompanied by a certain amount of loss in total brain weight.

III. EXERCISE EXPERIMENTS

1. Previous experiments on the effect of exercise upon the albino rat

Several investigators have worked upon problems connected with the changes in the albino rat occasioned by an increased amount of exercise. J. R. Slonaker in 1907 published observations upon four rats of different ages kept in revolving cages for a short period. In 1912, the same author published an account of further experiments along the same line, and although this time, also, the work was with a small group of rats, yet the experiment was continued during the natural life of the animals. Slonaker was working chiefly upon the problem of normal activity in its relation to age and sex but, incidentally, he made some few observations upon the comparative development of 'exercised' and normal rats. He found that "exercised rats are more active, more alert, and brighter in appearance than the control ones," but that "the control males reach their maximum weight at an earlier age than exercised males, and also greatly excel them" and that "control rats live longer than exercised rats." No observations were made on the effect of exercise upon any of the internal organs.

Donaldson, in 1911, conducted a series of experiments to ascertain the effect of exercise upon the central nervous system

of the albino rat, using the same sort of apparatus—a revolving cage with cyclometer attachment—employed by Slonaker. He found that there was a slight increase in brain weight (2.4 to 2.7 per cent) to be attributed to the effect of exercise. This was what was to be expected in view of the heavier brain to be found in the wild Norway rat. The cord showed no effect. The olfactory bulbs were not weighed separately.

Hatai ('15) published a series of observations based upon his own experiments and upon those of the present writer, showing the rather marked effect of the same exercise conditions upon the weight of the internal organs. In these experiments, the brains of the test animals showed an excess of 4 per cent over the controls with no effect upon the cord.

2. Description of Experiments. Series D and E

As it had thus been demonstrated that the brain of the albino rat could be modified by exercise in the revolving cage, it remained to determine whether, under such conditions, the olfactory bulbs would show a more marked variation than the brain as a whole.

For this work, also, large litters of stock albinos, were chosen. Each litter was weaned and divided into three groups when about thirty-five days old. One group constituted the 'Initial Controls,' and these were killed and examined as in the previous experiments. The second lot, the 'Final Controls,' was set aside in cages under the normal living conditions of the colony. The third group was used for the experiment. Each of these test animals was placed by itself in a wire revolving cage such as had been used by Slonaker, and later by Donaldson and Hatai. Each cage was 5 feet in circumference with an open nest box fastened to the central fixed axis. From this axis the food was suspended so that, theoretically, the rat must descend to the floor of the cage to eat. Practically, some rats soon learned to avoid this and so escaped a considerable amount of enforced exercise.

Each cage was provided with a cyclometer. Readings were made and recorded six times a week. These cyclometer read-

ings showed only the activity of the rats when the cage revolved and were therefore incomplete, since some rats learned to play from side to side of the cage and keep it from revolving, while others learned to run up the middle of the sides in such a way as to hold the cage at rest. But most of the rats soon learned to run the cages and appeared to enjoy it.

The rats were fed on the same diet as the controls and all the animals were weighed at intervals of about two weeks.

3. Series D. Rats in revolving cages for thirty days

There were but two litters in this series. One litter was weaned and set aside at thirty-five days of age and the other at forty days. Both litters were subjected to exercise in the revolving cages for a period of only thirty days. All were killed at the end of the thirty days of exercise.

a. Results. The exercised males of these two litters gained more rapidly in both weight and body length than did the controls, while the females fell behind. The superior growth of the test males was sufficient to bring the averages for both males and females up to 113 per cent of the weight of the controls and to 104 per cent of the length (tables 13 and 14).

The records of the activity of Series D were accidentally destroyed, but as these were for a period of but thirty days, they would be of little value save in adding further evidence that the female rat becomes active sooner than the male.

While, on the average, there is no difference in the absolute brain weight of the test rats in Series D from that of the controls, when both are compared with the reference table values in The Rat (Donaldson, '15), according to the method there suggested (pp. 4 and 5), yet I believe the bulbs do show, even after this short period, some effect of the unusal activity (tables 13 and 14). In the females, the bulbs make up 4.46 per cent of the brain weight in test rats as compared with 4.36 per cent in the controls. With the males, the difference was more marked—4.55 per cent in tests to 4.20 per cent in controls, making a joint average for males and females of 4.51 per cent in tests against 4.32 per cent

TABLE 13. SERIES D

Test animals

Albino rats kept in revolving cages for thirty-three days after weaning

Males

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS: PER CENT BRAIN WEIGHT
	days	*grams*	*mm.*	*grams*	*grams*	
PR_1	70	88	148	1.489	0.019[1]	1.30
T_3	68	131.7	170	1.871	0.079	4.21
T_2	68	140.9	176	1.831	0.085	4.63
PR_3	70	173.2	185	1.784	0.085	4.79
Average males		148.6	177	1.829	0.083	4.55
Females						
T_1	68	102.9	158	1.744	0.079	4.53
PR_2	70	116.0	162	1.653	0.073	4.39
Average females		109.5	160	1.698	0.076	4.46
Average males and females		132.9	170	1.776	0.080	4.51

[1] Lungs infected. Rat undersized in every way, therefore not included in averages (Series C, Sick rats, p. 218).

in controls, the olfactory bulbs of the former being, therefore, 7 per cent heavier than those of the latter.

4. Series E. Rats in revolving cages for ninety-eight to one hundred and three days

The test animals of this group were kept in the revolving cages for fifteen weeks. At the end of that time, three pairs of test animals and one pair of controls were mated (brother to sister in each case). Some digestive trouble appeared in the cages of control rats rather early in the experiment and most of the rats died, while the remaining animals failed to attain a normal growth, so that satisfactory final controls were lacking for this group. But the rest of the test animals and the surviving controls were killed at the end of the fifteen weeks, measured,

TABLE 14. SERIES D

Final controls

Males

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS: PER CENT BRAIN WEIGHT
	days	*grams*	*mm.*	*grams*	*grams*	
PR_5	70	100.7	154	1.636	0.061	3.72
PR_6	70	90.0	155	1.538	Bulbs unequal[1]	
T_4	68	132.4	170	1.802	0.084	4.64
Average males		116.6	162	1.719	0.072	4.20
Females						
T_5	68	110.8	159	1.780	0.081	4.52
T_6	68	115.6	165	1.774	0.077	4.32
PR_7	70	130.5	170	1.704	0.074	4.32
Average females		119.0	165	1.753	0.077	4.39
Average males and females		118.0	164	1.739	0.075	4.32
Test / Control			103.7%	102.1%	106.8%	

[1] Lungs slightly infected. Not included in average.

weighed, examined, and bulbs preserved exactly as in the underfeeding experiments.

The mated animals were kept about one hundred days longer to see whether the exercise of the previous weeks would show any effect upon fertility.

a. Results. General body growth. We find by examination of the records of body weight taken at two-week intervals during the experiment, that up to the time the larger set of control rats fell sick, the exercised animals were gaining less rapidly in weight than were the controls. From the time of the illness, some five weeks after the beginning of the experiment, the control rats fell off in weight, and with a single exception, they never recovered. Litter W escaped the infection and the weight records for the six rats composing it are as follows:

TABLE 15

Record for Litter W, Series E, showing gain in weight for individuals in revolving cages and for controls

	TEST RATS			CONTROL RATS		
	W_6 (m)	W_4 (f)	W_1 (f)	W_2 (m)	W_3 (m)	W_5 (f)
Initial weight.....	44.0 g.	40.5 g.	50.5 g.	43.5 g.	50.0 g.	40.5 g.
2 weeks weight.....	60.4	62.0	65.6	63.2	68.7	69.2
4 weeks weight.....	97.0	82.0	103.0	107.0	120.0	89.0
6 weeks weight.....	139.0	124.0	142.2	139.0	148.8	119.0
9 weeks weight.....	186.0	142.0	170.5	194.0	212.0	148.0
30 weeks weight.....	210.0	148.0	187.0	205.0	224.0	150.0
Final length.....	209 mm.	182 mm.	198 mm.	207 mm.	200 mm.	190 mm.

The test rats from Litter W were, on the whole, slightly longer and lighter in weight than the control animals. The majority of individuals in Litter W proved to have abnormal brains—one or both olfactory bulbs being very much undersized. The brains, therefore, could not be used for comparison and the litter was excluded from the tables. For comparison with the rest of the litters of Series E, it was necessary to use other stock litters, as will be described later (tables 18 to 25). The comparisons are not, therefore, of as much value as they would be were the controls from the same litter. On the average we find body length slightly more, and body weight slightly less, in test animals (table 25). I think we may conclude that these results agree in general with those of previous investigators indicating that exercise has but a slight effect, if any, upon either body weight or body length.

The size of the viscera was considerably modified. These results have been incorporated in the report by Hatai ('15).

Activity of exercised animals. These rats showed great individual difference in the amount of activity and in the age at which they became most active (tables 16, 19, 21, 25). In these respects, there was also a considerable difference in litters as shown by the following record.

If we take the record of these same rats for ninety-three days we get an average of 5.76 miles per day for males, and 5.96 miles

TABLE 16

Activity record of rats in revolving cages for one hundred and three days. Series E

ANIMAL	TOTAL MILES	MILES PER DAY	ANIMAL	TOTAL MILES	MILES PER DAY
Y_7M	914.5	8.9	Y_1M	559.7	5.4
Y_8F	770.5	7.5	Z_3M	476.3	4.6
Y_6F	724.0	7.0	X_6M	470.8	4.6
Y_4F	705.3	6.8	X_2F	458.7	4.5
Y_3M	689.0	6.7	Z_6F	457.1	4.4
Z_5M	577.8	5.6	Z_4F	446.6	4.3
Average for males...	614.7	5.96			
Average for females	593.7	5.76			

for females, and if we go back still further we get a still higher average for the females and lower for the males. The males were slow to begin to run the cages. An extreme example, Y_1 of the present series, ran less than 2 miles during the first five weeks in the cage, but became extremely active during the last four or five weeks making a final average of 5.4 miles per day, a record almost equal to the average for the entire lot of males. The females soon learned to run the cages and became very active at an early age. During the last weeks of the experiment, the activity of practically every female in the series was on the decline. I think from a study of all the records it may be concluded that while, in the revolving-cages, the females reach the period of greatest activity earlier than do the males, yet in the long run, the records of a large number of males and females would average about the same.

Possible effect on fertility. There is some indication that the fertility of the albino rat is increased by exercise. In the cases of the three pairs of exercised rats which were mated, the following record of offspring was obtained, together with the record of one control pair.

The average size of litter for normal stock albinos has been found to be between 6 and 7 individuals (Donaldson '15). This is about the average for the control pair, while the averages for the three test pairs is considerably higher—13, 10.5, and 9.

TABLE 17

TEST PAIRS	CONTROL PAIR
W_1 21st day after mating, 12 young and 61st day after mating, 11 young W_6 102d day after mating, 16 young pregnant	W_8 24 days after mating, 3 young and 50 days after mating, 12 young W_2 102 days after mating, 0 young not pregnant
Y_6 22d day after mating, 9 young and 67th day after mating, 9 young Y_3 102d day after mating, 0 young not pregnant	
Z_4 22d day after mating, 12 young and 88th day after mating, 9 young Z_3 102d day after mating, 0 young not pregnant	

It is significant also that the pair making the record of an average of 13 per litter for three successive litters, and the control pair are from the same original litter. Of course the numbers here are too few to enable one to draw conclusions but it would not be surprising to find some correlation between the greater weight of the sex organs in the exercised rats (Hatai '15) and the fertility of these animals.

Effect on brain and olfactory bulbs. It has already been noted that most of the control rats of this series were lost through disease. For comparison with the exercised rats, a set of controls used in Series A of the defective feeding experiment was chosen (table 20). These rats seemed better suited for the purpose than any others because they had been born at the same season as the test animals and reared in the same laboratory, so the food from day to day was the same for the two sets of rats. Among these, it was possible to find records of eight rats of almost the same body length and weight and of approximately the same age as the exercised rats killed at the end of the experiment. For the four which were mated and not killed until they were two hundred and thirty-eight days old, it was not possible to get controls of the same age, the four oldest of the controls (table 22) averaging only one hundred and sixty-nine days and the body length being 6 per cent less than that of the test animals. But as these are beyond the one hundred and fifty day limit, up to which time the bulbs increase in rel-

ative weight, the difference is not so serious a matter as it would be were the rats younger.

The set of test animals killed at the end of one hundred and three days of exercise, gave bulbs averaging for the males 4.28 per cent of the entire brain weight, and 4.60 per cent for the females—an average of 4.41 per cent for the entire set (table 19). When these results are compared with the controls (table 20) we find that while the test animals were 1 per cent shorter than the controls and had brains 2 per cent lighter in weight, the olfactory bulbs were 3 per cent heavier. These results seem to indicate that the olfactory bulbs of the test animals have been affected by exercise.

An examination of the records for the initial controls of the litters concerned seems to give additional weight to this supposition. See table 23 below.

TABLE 18. SERIES E

Initial control animals

Males

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS: PER CENT BRAIN WEIGHT
	days	*grams*	*mm.*	*grams*	*grams*	
W_1	30	26	99	1.338	0.052	3.89
Y_{10}	30	36	104	1.254	0.052	4.18
W_7	30	41	109	1.472	0.056	3.83
Z_8	30	41	112	1.444	0.050	3.46
Z_7	30	46	121	1.452	0.056	3.87
Average males		38	109	1.392	0.053	3.84
Females						
Y_5	30	29	96	1.21	0.043	3.57
X_5	30	24	96	1.280	0.045	3.52
X_1	30	28	101	1.338	0.034	2.51
W_6	30	34	107	1.335	0.043	3.22
W_5	30	43	114	1.432	0.048	3.34
Average females		31	103.	1.319	0.043	3.22
Average males and females		35	106	1.356	0.048	3.54

TABLE 19. SERIES E

Test animals

Albino rats kept in revolving cages for one hundred and three days after weaning

Males

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS: PER CENT BRAIN WEIGHT	AVERAGE NUMBER MILES PER DAY
	days	*grams*	*mm.*	*grams*	*grams*		
X_6	134	233	193	1.927	0.079	4.12	4.6
Y_1	135	188	195	1.693	0.070	4.16	5.4
Z_5	135	226	198	1.849	0.080	4.30	5.6
Y_7	135	237	205	1.875	0.085	4.54	8.9
Average males		221	198	1.836	0.079	4.28	6.1
Females							
X_2	134	151	177	1.661	unequal		4.5
Y_8	135	157	181	1.655	0.081	4.80	7.5
Z_6	135	162	180	1.796	0.078	4.32	4.4
Y_4	135	169	191	1.693	0.079	4.64	6.8
Averages females		162	184	1.715	0.079	4.60	5.8
Average males and females		196	192	1.784	0.078	4.41	5.95

As we see, the brains of the initial controls for the test animals (X, Y, Z) averaged but 91 per cent of the weight of the initial controls for the final controls (L, N, O, T, U, V); the olfactory bulbs but 89 per cent. Since it has been found that brain and olfactory bulb weight are pretty uniform for any given litter, and that when we find light or heavy brains or bulbs in the initial controls, we are fairly sure of finding the same relative development in the adult animals of the same litters, it seems fair to assume that normal adult individuals of litters X, Y, Z, would have had relatively lighter brains and bulbs than were found in adults of litters L, N, O, T, U, and V. If this assumed relation were true, then the results given in tables 19 and 20 doubtless would fall into line with those of previous experiments in which exercised rats showed an increase in brain weight over the

TABLE 20. SERIES E

Final control animals[1]

Males

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS: PER CENT BRAIN WEIGHT
	days	*grams*	*mm.*	*grams*	*grams*	
T_6	160	223	202	1.890	0.085	4.51
U_3	146	228	202	1.987	0.081	4.06
N_8	121	203	205	1.825	0.072	3.93
L_6	157	274	218	2.021	0.077	3.81
Average males		232	206	1.931	0.079	4.08
Females						
H_8	93	127	172	1.559	0.063	4.03
V_7	124	144	177	1.672	0.067	4.03
T_7	213	183	187	1.801	0.082	4.56
O_1	115	175	188	1.792	0.083	4.62
Average females		157	181	1.706	0.074	4.32
Average males and females		195	194	1.818	0.076	4.20
Series A $\frac{\text{Test}}{\text{Control}}$			99%	98%	103%	

[1] Data from stock Albinos used for controls in Defective Feeding Series A and again used for comparison here, since the original controls died early in the experiment.

controls, and would indicate an even greater gain in bulb weight for the test animals than is indicated in the tables.

In the same way, we may compare the initial controls for the mated test animals and those for Series A used for a standard (tables 21 and 22). We find the initial relations practically the same as for the group just discusesd.

In the final results (tables 21 and 22) we see that although the test rats were older, with bodies 6 per cent longer, the brains were actually 5 per cent lighter in weight. Here again, examination of the initial controls suggests that in all probability there was not an actual loss of brain weight in the exercised animals.

TABLE 21. SERIES E

Test animals

Albino rats kept in revolving cage for one hundred and three days after weaning. At end of that time mated and allowed to rear 2–3 litters. Age, when killed, about eight months.

Males

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS PER CENT BRAIN WEIGHT	AVERAGE NUMBER MILES PER DAY
	days	*grams*	*mm.*	*grams*	*grams*		
Z_3	238	299	221	2.018	0.092	4.54	4.6
Y_3	238	311	228	1.842	0.094	5.10	6.7
Average males		305	225	1.930	0.093	4.81	5.7
Females							
Z_4	238	216	202	1.777	0.080	4.58	4.3
Y_6	238	156	203	1.654	0.080	4.81	7.0
Average females		186	203	1.716	0.080	4.66	5.7
Average males and females		246	214	1.823	0.086	4.74	5.7

TABLE 22. SERIES E

Control animals

Stock albinos used for control in defective feeding experiment, Series A. The four oldest of this set chosen for present tests since original controls died early in the experiment.

AVERAGE	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS PER CENT OF BRAIN WEIGHT
U_3	146	228	202	1.987	0.081	4.06
L_6	157	274	218	2.021	0.077	3.81
T_6	160	223	202	1.890	0.085	4.51
T_7	213	183	187	1.801	0.082	4.56
Average		227	202	1.925	0.081	4.23
Series A $\frac{\text{Test}}{\text{Control}}$			106%	95%	106%	

TABLE 23

INITIAL CONTROLS FOR TEST ANIMALS				INITIAL CONTROLS FOR CONTROL ANIMALS. (DEFECTIVE FEEDING EXPERIMENT)			
Litters	Average brain weight	Average olfactory bulbs weight	Per cent of brain weight	Litters	Average brain weight	Average olfactory bulbs weight	Per cent of brain weight
	grams	*grams*			*grams*	*grams*	
X, Y, Z.....	1.305	0.047	3.60	L, N, O, T, U, V.	1.431	0.058	3.69
Test / Control	91%	89%					

TABLE 24

INITIAL CONTROLS FOR TEST ANIMALS				INITIAL CONTROLS FOR CONTROL ANIMALS (DEFECTIVE FEEDING EXPERIMENT)			
Litters	Average brain weight	Average olfactory bulbs weight	Per cent of brain weight	Litters	Average brain weight	Average olfactory bulbs weight	Per cent of brain weight
	grams	*grams*			*grams*	*grams*	
X and Y.....	1.340	0.051	3.76	L, U and T	1.458	0.055	3.76
Test / Control	92%	92%					

But, be this as it may, we find the bulbs of these test animals actually 6 per cent heavier than those of the controls, the bulbs making 4.74 per cent of the total brain weight, while those of Series A controls were only 4.23 per cent of the total weight of the brain.

Since we have no true control series for comparison, we can not, of course, draw conclusions as to the absolute gain in brain weight after exercise. But of the gain in olfactory bulb weight in exercised animals, there seems to be no doubt.

When we turn to table 25 and note that the average percentage weight for the bulbs in case of 29 normal rats (59 to 83 days old) is 4 per cent, while a study of table 13 shows there was no rat there recorded (save one sick one) in which the per cent fell below 4.21 per cent, while the average was 4.51 per cent, we must be convinced, I believe, of the reality of the effect of exercise. For the older rats, likewise, when we compare tables 19 and 25,

TABLE 25

(Summary) Showing comparative development of the olfactory bulb

Stunted series

TABLE	NO.	HISTORY	AGE	BODY WEIGHT	BODY LENGTH	AVERAGES FOR BRAIN WEIGHT	BULBS WEIGHT	BULBS PER CENT BRAIN WEIGHT	RATIO AVERAGE TEST TO AVERAGE CONTROL IN			AVERAGE MILES PER DAY
									Body length	Brain weight	Bulbs weight	
			days	*grams*	*mm.*	*grams*	*grams*		*per cent*	*per cent*	*per cent*	
II and XVIII	38	Initial control. Underfed and Exercise Experiment	3; 18–20 35; 29–34	40	111	1.400	0.050	3.54				
X	9	Underfed from birth	27–53	18	83	1.091	0.034	3.14				
XI	3	Underfed from birth	77	31	102	1.437	0.050	3.48				
III	26	Underfed 29–32 days	59–62	57	126	1.504	0.053	3.52	77	91	81	
IV	26	Controls for above	59–62	119	164	1.651	0.066	3.99				
V	3	Underfed 49 days	78–83	44	124	1.487	0.050	3.39	69	87	70	
VI	3	Controls for above	78–83	154	180	1.719	0.072	4.16				
VII	13	Underfed 59–130 days	93–160	51	122	1.524	0.058	3.83	63	85	77	
VIII	12	Controls for above	93–160	187	193	1.772	0.076	4.30				
		Sick animals										
XII	3	Bowel infection	134–135	146	176	1.647	0.050	3.02				
XII	8	Infected lungs	70–370	187	190	1.742	0.034	1.96				
		Exercise series										
XIII	6	Revolving cage 30 days	68–70	133	170	1.776	0.080	4.51	103.7	102.1	106.8	
XIV	6	Controls for above	68–70	118	164	1.739	0.075	4.32				
XIX	8	Revolving cage 103 days	134–135	196	192	1.784	0.079	4.41	99	98	103	5.95
XX	8	Controls for above	93–213 (av. 141)	195	194	1.818	0.076	4.20				
XXI	4	Revolving cage 103 days and mating	237	202	214	1.823	0.086	4.74	106	95	106	5.65
XXII	4	Controls	146–213 (av. 169)	227	202	1.925	0.081	4.23				

we see that the average for 12 controls (90 to 160 days old) was 4.26 per cent while only two test animals fell as low as this (one of these was of abnormally light body and brain), and the averages were 4.41 per cent and 4.74 per cent for four and one-half months and eight months respectively.

5. Summary

1. The results of the present experiments agree with those of previous investigators in that they show no marked effect of exercise either upon body length or body weight in the albino rat.

2. The female albino becomes very active earlier than does the male but the activity of the male later increases to such an extent that the total activity for the two sexes for long periods is probably about equal.

3. These experiments suggest that there is an increase in fertility correlated with increase in the size of the reproductive organs.

4. The brain weight is slightly increased by exercise.

5. The weight of the olfactory bulbs of albino rats exercised in revolving-cages for periods of from thirty to one hundred days, is considerably increased. The bulbs of such rats form from 4.41 to 4.74 per cent of the total brain weight as compared with 4.20 to 4.32 per cent in rats reared under normal colony conditions. These bulbs show an increase of 5 to 11 per cent over and above the increase in weight manifested by the entire brain.

IV. CONCLUSIONS

From the preceding observations we may conclude that we are able to modify the olfactory bulbs of the rat by changing the conditions under which it lives and to modify them to a considerably greater degree than we can change the rest of the brain. In cases of stunting, the bulbs tend to overcome the effect, to a certain extent, as time goes on. With exercise the effect seems to increase with age. Yet the bulbs respond more markedly to the stunting effect of defective feeding or sickness than to the stimulating effect of exercise.

A histological study of these modified bulbs will be presented in the second part of this paper.

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PART II. ON THE NUMBER OF NERVE CELLS IN LARGE AND SMALL OLFACTORY BULBS

CONTENTS

I. INTRODUCTION

The foregoing studies have shown that it is possible to change the relative weight of the olfactory bulbs in the albino rat (Holt, '17). This relative weight is decreased by a defective diet and increased by exercise. Such being the case, it seemed very desirable to make a histological comparison of the bulbs which had been stunted by a defective diet or enlarged by exercise, with those of rats reared under normal colony conditions. For this purpose it was of course essential to find a method of fixation and treatment which would give uniform results. Fixation in Ohlmacher's solution as recommended by King ('10) for the study of cortex cells, gave very satisfactory results, but her statement that "various individuals react differently although subjected to the same course of treatment," and her tables (loc. cit., p. 231) showing a variation in shrinkage ranging from 2 to 18 per cent in brains so fixed, suggested that it would be best to examine this method a little more in detail. Unless uniform results could be obtained by it, this method would, of course, be un-

suited to comparative study of the size of bulb elements. Accordingly, the method was further tested, and at the same time an examination was made of the effect of Müller's fluid and of Orth's Formol-Müller solution upon the various parts of the brain.

A long series of experiments demonstrated quite conclusively the following points which have an important bearing upon the present investigation.

1. Of the three fixing fluids tested, Ohlmacher's solution causes the least change in weight in brain tissue.

2. Orth's solution (cold) causes a slight increase in weight.

3. Müller's solution causes a very considerable increase in the weight of brain tissue as has already been noted (Donaldson, '94).

4. Olfactory bulbs, fixed in Ohlmacher's solution, reach a state of equilibrium at the end of twenty-four hours; fixed in Formol-Müller, they reach this state at about the end of one week; fixed in Müller's solution alone, changes continue from six weeks to two months.

5. There seems to be no appreciable individual variation in the reactions of albino rat brains of like age to Ohlmacher's solution, to Müller's fluid, or to the Formol-Müller solution. The results obtained by Dr. King are due apparently to the fact that the brains which were weighed in her experiments had been fixed for varying short lengths of time and the initial changes in weight were so rapid that there appeared to be a considerable difference in the way the various brains reacted to the fixative, when in reality, had all the brains been fixed for exactly the same length of time, no such large disagreement would have been found.

II. THE PROBLEM OF SIZE DIFFERENCES

Although under normal conditions, there is a good deal of variation in the size of the olfactory bulb of the albino rat, we have found that it is possible, experimentally, to increase this range of variation to a very considerable degree. The question next arises as to the structural cause of the difference

in size. Is it one of size of elements or of their number? Have we more cells and fibers in the heavier bulb or are the cells and fibers merely of a larger size? The present paper deals only with the question of cells. The fibers have yet to be examined.

III. TECHNIQUE AND METHODS OF STUDY

Since experiments on the effect upon the rat brain, of Ohlmacher's, Müller's, and the Formol-Müller solutions have demonstrated that for brains of like ages there is a definite, practically unvaried, swelling or shrinking reaction for any given fluid, the brains of the rats used in the defective diet and exercise experiments were fixed in these several solutions for histological comparison. For the present cell study the method adopted was that recommended by King ('10) for study of the cortex, namely fixation in Ohlmacher's solution for twenty-four hours, followed by one hour in 85 per cent alcohol, three to four days in iodized 70 per cent alcohol, double embedding in celloidin and paraffin, and staining in carbol-thionin and eosin. However, after the first trials, this method was varied in the matter of embedding. For such small objects as the olfactory bulbs, paraffin proved more satisfactory when used alone. Sections were cut 8μ thick and mounted serially. A rather deep thionin stain gave the best results for cell enumeration.

1. Preparation of sections

At first, some bulbs were cut sagittally and the largest sections compared. In the study of these sections the number of cells in the gray layer of the different bulbs was found to be so nearly identical that it was decided to attempt a thorough study of cell number.

In dissecting a rat brain into its parts, the bulbs are cut from the brain in such a way as to leave an appreciable portion of the bulb attached to the cerebrum. The method followed was to place the brain, ventral side down, on a flat surface and with a knife held in a plane perpendicular to the table, to sever the bulb at the point where it disappears beneath the cerebrum (plate 1).

This is the part of the bulb which is weighed, and since all bulbs are removed in the same way, it has been assumed that we have corresponding portions for comparison. Because the recorded weights represented only this portion of the bulbs, it seemed advisable at first to compare the cell elements of these parts only. Accordingly cross sections of the bulbs were made but unfortunately, as appeared later,most of the test series were not complete for the portion of the gray substance beneath the hemispheres. The method of meeting this difficulty will be described later.

2. Methods of study

The study of sections was made largely with the aid of the Edinger projection apparatus. Cell counts were made by projecting the sections onto white wrapping paper, outlining the area, and punching the image of each cell nucleus with a tallying register fitted with a sharp prong in place of the usual blunt register arm (Hardesty '99). The hole punched by this prong insured against counting the same cell twice. It also left a permanent record of any particular region which could be reexamined later. In some cases the count for each section was recorded; in others, the whole number of sections to be counted were registered consecutively and no record made until the end. Occasionally a section was recounted—to serve as a check on the work.

IV. GENERAL DIFFERENCES IN SIZE

Most of the comparisons of size and the determinations of cell number have been made on bulbs stunted by a defective diet, and on their respective controls. Only two bulbs of the exercise series have yet been examined.

The general differences in size between young and mature, stunted and normal olfactory bulbs are very well illustrated by the sections shown in Plates 1, 2, and 3. Figure 1 of plate 1 is a camera drawing of a median sagittal section through the bulb of a rat stunted by feeding for thirty-one days on a corn diet. The body length was 138 mm.; brain weight, 1.547 grams and the weight of the corresponding bulb which was removed and

weighed, was 0.020 gram—the approximate weight then of the bulb shown in the drawing. Figure 2, plate 1, is a median sagittal section through the control bulb. The rat from which this was taken was 166 mm. long, with brain weight of 1.698 grams, and the weight of the corresponding bulb was 0.032 gram. Like the bulbs of very young rats, the gray layer of the stunted bulbs extends somewhat further, in proportion, beneath the cerebrum than in the case of normal older individuals.

When the stunted bulb is compared with its control, there appears to be a rather uniform size difference involving all parts of the bulb. It is hard to compare the outer fiber layers owing to the difficulty in removing the bulbs perfectly from the skull. The anterior end of the fiber layer is very likely to be entirely torn away and sometimes the ventral side also suffers. However, it is plain that the glomeruli of the larger bulb are much larger and more open; the granular cells are not so closely packed together; and the gray layer is usually broader in the larger bulb and the inner granular area considerably more extensive. These differences between the peripheral portions are illustrated by the more highly magnified mid-dorsal areas *S* and *S* of figures 1 and 2, shown in figures 3 and 4 of plate 2.

Plate 3 shows three cross sections; through Q_5, a thirty-day control bulb (fig. 5); M_4, a sixty-two-day stunted bulb (fig. 6); and M_5, the sixty-two-day normal control (fig. 7), for M_4. These sections were made through the region where the bulbs are usually cut from the brain. The figures illustrate another typical difference. The normal bulb (figs. 5 and 7), as it grows, elongates more rapidly than it increases in thickness and it tends to grow faster dorso-ventrally rather than laterally. In these figures, the outer fiber layer is probably complete at the sides. Ventrally it has doubtless been torn away to some extent in all three bulbs. The difference in size of the glomeruli is well shown here, but while there is a greater area of gray matter in figure 7 than in the other two, the gray layer seems narrower than in M_4 (fig. 6). The companion bulb of Q_5, (fig. 5) weighed 0.024 gram, that of M_4 (fig. 6), 0.025 gram while M_5 weighed 0.037 gram (fig. 7). The portion of Q_5 anterior to the section

illustrated, was about 1350μ long, while M_4 had 1500μ anterior to the section, and M_5, 2000μ. The differences in size are confined to no one region but are distributed somewhat proportionally through the different layers.

V. COMPARISON OF CELLS OF GRAY LAYER

1. Size and number of small cells in molecular layer

It has been the general impression that, within certain limits, the size and weight of the brain are indices to its functional capacity. In the phylogenetic series, of course, it is, with one or two exceptions, true that increase in intelligence is accompanied by increase in the relative size of the brain. So within any given species of mammals, it has been assumed that the more efficient brain is the larger and heavier.

The question as to whether, within such a group, increase in size of the brain means an increase in the number of nerve elements or in the size of the elements themselves, becomes an important one. For an increase in the number of elements should give increased functional possibilities. So, if we find in comparing large and small brains or divisions of brains from closely related animals, that the larger structure contains a greater number of cells and fibers, then we have reason to expect from the larger and more complex structure the greater degree of efficiency.

If, on the other hand, the number of elements is found to be uniform for the part under consideration, then we must conclude that the large and the small brains have potentially the same ability to function, save as their efficiency may be affected by the size or degree of development of the individual elements.

The small cells of the molecular layer (*mo*, fig. 2) show more cytoplasm; or perhaps we may say that it is possible to distinguish more cells with cytoplasm in the molecular layer of large bulbs than of small ones. For example, the section of F_1 shown in figure 1 shows 68 cells between mitral layer and glomeruli, in which cytoplasm may be distinguished, while the control, F_5, shows 158 such cells. Corresponding sections through M_1,

a thirty-day control, and C_3, a sixty-day underfed bulb, show 108 and 103 cells with cytoplasm.

Although a difference in cell size appeared, there seemed to be little difference in numbers of cell elements in the gray layer. Although it is not always possible to distinguish between the nuclei of very small cells and possible cross sections of fibers under the conditions used for counting—yet the error due to this difficulty is probably negligible. A preliminary count was made of all elements, having the appearance of nuclei in the largest sections of the bulbs F_1, C_3, F_5, F_6, and M_1, with the following results.

TABLE 1

INITIAL CONTROL				TEST				FINAL CONTROL			
Bulb	Age	Bulb weight	Number cells	Bulb	Age	Bulb weight	Number cells	Bulb	Age	Bulb weight	Number cells
	days	*grams*			*days*	*grams*			*days*	*grams*	
M_1....	30	0.029	2172	F_1	62	0.020	2569	F_5	61	0.032	2569
				C_3	59	0.021	2604	F_6	61	0.033	2693

These counts for the test and final control bulbs suggested so strongly that the number of cells is the same for bulbs of different sizes that attention was turned entirely to the investigation of this point. At first longitudinal sections were used, but these were soon abandoned for two reasons. First, it seemed desirable to be able to count the cells of just that portion of the bulbs corresponding to the part weighed; and second, the longitudinal sections presented so many irregularities that it was necessary to count many more sections to approximate the true average than in the case of the cross sections. Counts were made of all elements in the gray layer outside the mitral layer between the tip of the bulb and the point at the proximal end where the gray layer is first interrupted on the dorsal aspect of the bulb (see figs. 5, 6, 7). These counts consumed a vast amount of time and when completed seemed to disagree with the observations already made upon the longitudinal sections (table 2).

A first glance at the table would indicate that the small bulb has fewer cells and would suggest that this difference in cell

TABLE 2

BULB	AGE	HISTORY	BRAIN WEIGHT	WEIGHT 1 BULB	NUMBER SMALL CELLS
	days		*grams*	*grams*	
X_1....	30	Control	1.338	0.017	636,656
M_3....	62	Underfed 31 days	1.461	0.025	662,982
G_{12}....	62	Underfed 31 days	1.543	0.027	601,982
G_6.....	61	Normal control	1.630	0.031	675,305
M_5....	62	Normal control	1.711	0.037	716,582
X_6....	134	Revolving cage 104 days	1.927	0.040	789,680

number is one of the factors in bulb size. But corresponding sagittal sections had given fairly close agreement in numbers and the study of sagittal sections made it more and more evident that these counts of cross sections could be taken only to compare the parts commonly considered the bulb and not for an enumeration of the cells in the whole bulb. The difference in shape in the large and small bulbs made it apparent that a true count must be made either from sagittal sections or from cross sections cut through the entire length of the gray matter covering the bulb. Comparison of such sections as figures 1 and 2 made it clear that if we had, in reality, a constant number of cells in the gray layer, the numbers in the regions here designated as the 'bulb' could scarcely be expected to show any closer agreement than we find in this table, and would probably have the relations there given. For the larger and better developed the bulb, the greater the proportion of it lying anterior to the cerebrum, while the young or the stunted bulb runs somewhat further back beneath the hemisphere and so some of the cells escaped enumeration. For example, M_5, a bulb of 0.037 gram, has 271 sections containing mitral cells in the portion of the bulb beneath the cerebrum. M_4, the test bulb of this litter, which weighed but 0.025 gram had 336 sections in this region. Taking these facts into consideration, the table in question pointed to a uniformity rather than variation in numbers corresponding to size. Later we shall see how, in the light of the study of the mitral layer, a part of this table can be shown to closely conform to this supposition that the number of cells in the entire gray layer is approximately constant for olfactory bulbs of different sizes.

2. Size and number of mitral cells

The cells of the mitral layer show a good deal of variation in size and shape and there is much difference in these respects in different regions of the same bulb. This makes the comparison of the size of the mitral cells in large and small bulbs rather difficult.

But if all the mitral cells of a section from a small bulb are drawn with a high magnification by means of camera lucida or projection apparatus and those cells arranged side by side with a series from a corresponding section of a large bulb, drawn to the same scale, it is possible to make a general comparison. In this way the mitral cells have been compared, and there is no doubt I think that the mitral cells of large bulbs are larger and better developed than those of small bulbs.

Details of technique and examination. It was sometimes quite difficult to determine whether a cell should be counted or not. For instance, when counting mitral cells it was hard to know at times whether a cell was a mitral cell or a brush cell, as many cells occur in the mitral layer which are exactly like those large cells occurring in the molecular layer but which lack the typical mitral form. On the other hand, typical mitral cells occur not infrequently out in the molecular layer or even among the granules on the inner edge of the glomerular layer. For this reason and in order that there might not be any unconscious influence in deciding whether cells should be counted, an attempt was made to vary the order of procedure for each successive count.

A rather complete count was made of the mitral cells of fourteen bulbs and of the small cells of the gray layer in four bulbs. Eight of these were cut longitudinally and six cut transversely.

The first series counted were those of X_1, Initial control, Series E and X_6, Test, Revolving Cage Series. In both series every other section was counted for the region anterior to the cerebrum—corresponding to the portions of these same bulbs in which the small cells of the gray layer had been counted. The result was 64,470 cells for X_1, a 0.017 gram. thirty-day control

bulb. The number for X_6, whose weight was 0.040 gram, was 73,950. To see whether there were any virtue in making so thorough a count of cross sections, the total number was computed from a recount of every 10th section, excepting at the most anterior end where every cell was counted in every section, until the sections showed a single layer of mitral cells. By this method the number obtained for X_1 was 64,775 cells, making a difference of only 0.4 per cent. For X_6 the count was 73,324, which was 0.8 per cent smaller than the more exact count obtained by counting half the sections. These differences were so small as to make the more exhaustive count seem unnecessary. X_6 gave an almost complete series through the entire gray layer so the count was completed, giving for the entire bulb 80,114 cells. The count of the mitral cells in X_1 could not be completed as the series had been cut, unfortunately, with the idea of comparing only the parts of the bulbs whose weights we knew, and which, therefore, extended back but a short distance under the cerebrum. The cells of these few sections were, however, counted, giving a total of 71,914.

The number of mitral cells in G_6 was computed from absolute counts of anterior and posterior ends of the series and by counting every tenth section through the rest of the series. M_4, M_5 and Q_5 ran so evenly that here in the middle portion of each series, only every twentieth section was counted; on either side of this portion, every tenth section, and all cells of all sections at either end.

With the sagittal sections, the task was more difficult and the results, I believe, less reliable for this reason: toward the sides of the bulbs, especially the median side, the sagittal series may give tangential sections of the mitral layer so that a single section may yield a count of 1500 cells whereas a section two or three removed on either side might have but 300 or so mitral cells. It can be easily seen that if the section to be counted, happened to fall in such a region, or entirely skipped such a region, the count would be considerably modified. Some of the bulbs gave no trouble of this kind while others were hard to count for this reason. G_3 was an interesting example of the way this

may work out. A count was first made of all cells at either end of the series and those in every tenth section through the middle portion. The result when computed was 95,993 mitral cells. Then the middle section of every ten was counted with a total result of 83,974 cells. Two other series were attempted but abandoned as the bulbs were so irregular that an accurate count would have required the enumeration of the cells of at least every alternate section. The other bulbs, except C_3 for which every fifth section was counted, were fairly regular so that the mitral layer offered no such complications. For these, the method of counting all cells at either end of the series and those of every tenth section through the median portion was followed. The sequence of counts was varied with each bulb, and the records kept in various ways and not infrequent recounts made. The recounts were surprisingly close to the original, for, as has been stated, it is not always easy to decide whether or not a cell should be counted, and in focusing as one counts, a granule lying below or above a portion of a mitral cell sometimes looks very like a nucleus, but the error due to this cause is probably too small to be considered.

Details of counts of the different bulbs, arranged in the order followed in table 3.

Bulb X_1, thirty day, initial control. Cross sections. Mitral cells counted in every section of anterior end back to the first section in which the mitral cells appeared in a single layer. From this point, counts were made for every tenth section back to the cerebrum. By computation, the total number of mitral cells was 64,775. By a recount in which the mitral cells of every other section were enumerated the computed number was 64,470 making a difference of only 0.4 per cent in the two counts. The series of sections for the region beneath the cerebrum was incomplete, the posterior portion not having been preserved. A count was made, however, of the sections which were present. This number added to the number already counted by the second method, brought the total up to 71,914 cells.

Bulb E_1, Test, defective diet series. Sixty-two days. Sagittal sections. Mitral cells counted in all sections at either end of the series and for every tenth section between.

Bulb C_3 Test, defective diet series. Fifty-nine days. Sagittal sections. Counts made as in E_1.

Bulb Q_5, thirty day, initial control. Defective diet series. Cross sections. Mitral cells counted in all sections at both ends of the series.

TABLE 3

Giving number of mitral cells in one olfactory bulb of the albino rat

Arranged according to bulb weight

RAT	AGE	BODY LENGTH	BRAIN WEIGHT	WEIGHT 1 BULB	NO. MITRAL CELLS	REMARKS
	days	*mm.*	*grams*	*grams*		
X_1 Control.....	30	101	1.338	0.017	71,914	Very incomplete. Up to point of union with cerebellum 64,470 cells, cross section
E_1 Test.....	62	138	1.547	0.020	71,527	Sagittal section
C_3 Test.....	59	142	1.482	0.022	79,165	Sagittal section
Q_5 Control.....	30	97	1.316	0.024	82,192	Probably about 300 more cells. Cross section
C_5 Test.....	59	150	1.578	0.024	70,625	Sagittal section
M_4 Test.....	62	109	1.461	0.025	76,611	Cross section
G_3 Test.....	61	136	1.456	0.027	83,974	Sagittal section
O_5 Test.....	115	121	1.556	0.029	71,663	Sagittal section
G_6 Control.....	61	166	1.630	0.031	81,638	Cross section
F_5 Control.....	61	166	1.698	0.032	71,468	Sagittal section
C_7 Control.....	62	174	1.709	0.036	79,839	Sagittal section
M_5 Control....	62	169	1.711	0.037	76,596	Cross section
X_6 R. C. Test..	134	193	1.927	0.040	80,114	Up to point of union with cerebrum 73,950 cells, cross section
O_1 Control.....	115	175	1.792	0.041	72,333	Sagittal section(X_1 omitted from average)
Average.....					76,749	

Standard deviation $\sigma = 4564$

Probable error of the mean ± 855

Through the middle region only every twentieth section was counted as the sections were extremely uniform. Through the two regions between this middle portion and the ends in which all cells were counted the mitral cells for every tenth section were counted.

Bulb C_5, test, defective diet series. Fifty-nine days. Sagittal sections. Mitral cells counted for all sections at both ends of the series and every fifth section of the rest of the series.

Bulb M_4, test, defective diet series. Sixty-two days. Cross sections. Mitral cells were counted as in Q_5. Count was made also of all cell elements in every alternate section in the gray layer back to the anterior end of the cerebrum. Computation was made for entire series.

Bulb G_3, test, defective diet series. Sixty-one days. Sagittal sections. Mitral cells were counted for all sections at ends of series and for every tenth between. The computed result was 95,993. The middle sections between every tenth were then counted, giving a

TABLE 4

Giving number of mitral cells in one olfactory bulb of the albino rat

Arranged by litters

AGE	RAT	WEIGHT 1 BULB	NUMBER MITRAL CELLS	PERCENTAGE DIFFERENCE OF TEST	REMARKS
days		*grams*			
62	F_1 Test	0.020	71,527 (S.)	+ 0.08	
62	F_5 Control	0.032	71,468 (S.)		
115	O_5 Test	0.029	71,663 (S.)	− 0.9	
115	O_1 Control	0.041	72,333 (S.)		
62	M_4 Test	0.025	76,611 (C.)	+ 0.2	
62	M_5 Control	0.037	76,596 (C.)		
59	C_3 Test	0.022	79,165 (S.)	− 0.9 \ Ave.	
59	C_5 Test	0.024	70,625 (S.)	−12.0 / −6.5	
62	C_7 Control	0.036	79,839 (S.)		
30	X_1 30 d. T.	0.017	71,914 (C.)		Very incomplete
134	X_6 R. C. T.	0.040	80,114 (C.)		Slightly incomplete (not in average)
61	G_3 Test	0.027	83,974 (S.)	+ 2.9	
61	G_6 Control	0.031	81,638 (C.)		
30	Q_5 30 d. C.	0.024	82,192 (C.)		Probably 300 more cells
Average per cent difference of test....				− 1.8	

(S.) = Sagittal section.
(C.) = Cross section.

count for every fifth section of this region. The computation then gave a total of 83,974. Two other bulbs of this litter were also cut in sagittal sections and an attempt was made to count the mitral cells but the bulbs were so irregular that it would have been necessary to count practically every section, so these counts were abandoned.

Bulb O_5, Test, defective diet series. One hundred and fifteen days. Sagittal section. Counts made as in E_1.

Bulb G_6, control, defective diet series. Sixty-one days. Cross section. Counts made as in E_1.

Bulb F_5, control, defective diet series. Sixty-one days. Cross section. Counts made as in E_1.

Bulb C_7, control, defective diet series. Sixty-two days. Sagittal sections. Counts as in E_1.

Bulb M_5, control, defective diet series. Sixty-two days. Cross sections. Counts as in M_4 and computation of all cell elements in gray layer made for entire series.

Bulb X_6, test, revolving-cage series. One hundred and thirty-four days. Cross sections. Mitral cells counted by both methods described for X_1. Also all cell elements of the gray layer computed for the entire series as in M_4 and M_5.

Bulb O_1, control, defective diet series. One hundred and fifteen days. Sagittal section. Mitral cells counted as in E_1.

Table 3 gives the results of the counts of the mitral cells of fourteen olfactory bulbs, arranged according to bulb weight. It is obvious that there is no correlation between bulb size and the number of the mitral cells or between age—within the limits taken—and number of cells. The numbers range from 70,625 to 83,974 with an average of 76,750 cells for 13 bulbs, X_1 being omitted from the average. I am inclined to think 83,974 cells is too high a count for G_3 and that still closer enumeration might yield a lower number. A recount was made for C_5 counting every fifth section, as this was a somewhat irregular bulb and it was thought that might account for the variation of this bulb from the rest of the litter. But the recount gave practically the original number.

Table 4 which is arranged by litters indicates a striking agreement between the members of the same litter. With the exception of litter C, in which C_5 falls 12 per cent below the control in number of mitral cells, there is extremely little difference between test and control bulbs of the same litter. So we find that whether the bulb has been stunted by a defective diet, or enlarged by exercise, the number of mitral cells is practically constant for any given litter. The factors which have brought about a change in size of the olfactory bulbs have failed to affect the number of mitral cells, at least in the gray layer. The test and control counts are, with one exception, extremely close. This is a fresh example of the similarity in structure among members of the same litter—a relation which is continually appearing in the study of this animal.

3. Study of the small cells in the gray layer

The sections of bulbs M_4, M_5, X_1, and X_6 are all series in which a count was made of the small cells of the gray layer in the anterior portion of the bulb as well as of the mitral cells. Assuming that the relation between the number of mitral cells in two given sections of a bulb would be the same as that between the small cells of the gray layer in these same regions, computation was made of the total number of small cells in the gray layer of M_4,

M_5, and X_6. Bulb X_1 was too incomplete to make such calculation possible. Let us take M_4. The small cells were counted back to section 4, 1/1. We have the total number of mitral cells and also the number of mitral cells back to section 4, 1/1. This gives us the data for computing the total number of small cells as follows:

Mitral cells of M_4 to section 4, 1/1 50,729
Total mitral cells 76,611
66 per cent of mitral cells in anterior portion.
Number small cells to section 4, 1/1 662,982

If this number equals 66 per cent of the total number, then the total number of small cells in the gray layer would be 1,004,518.

If we treat M_5 in the same way we have:

Mitral cells to section 3, 6/12 55,775
Total mitral cells 76,595
73 per cent of mitral cells in anterior portion.
Number small cells to section 3, 6/12 716,382
Then total number small cells 981,619

According to this computation the test bulb would have two per cent more cells than the control.

Now if we treat X_6 in like manner we have the following:

Mitral cells to section 5, 1/7 62,060
Total mitral cells 80,114
77 per cent of mitral cells in anterior portion.
Small cells to section 5, 1/7 789,680
Then total number of small cells 1,020,258

These total numbers are strikingly close. The number of bulbs is too small to warrant us in drawing general conclusions but I think the results certainly point to close agreement in number even of the small cell elements in the gray layer.

While there is a constant increase in the number of myelinated fibers correlated with age, as has been demonstrated by Greenman ('13) for the peroneal nerve, Boughton ('06) for the oculomotor, Hatai ('02) and ('03) for both dorsal and ventral roots of several spinal nerves, and Dunn ('12) for the ventral root of the second cervical nerve; there is very little evidence of any true increase in the number of cells in the central nervous system after the first few days after birth. Allen ('12) found dividing cells in the cerebellum up to twenty-five days and in the cerebrum up to twenty days, with a few along the lateral walls of

the lateral ventricles until the end of the second year. Hatai observed an increase in number of cells in the spinal ganglia, corresponding to increase in age but this increase was attributed in part, at least, to failure to count all the ganglion cells in very small animals. Ranson ('06) in a study of the second cervical nerve found no correlation between the number of cells and the number of myelinated fibers, neither did he find the number of cells to vary with the age of the rat.

The results of the present investigation of the number of cells in the olfactory bulb help to confirm the impression that the number of cells in the central nervous system becomes fixed at an early age so that after the first three or four weeks at least, there is no material change in the cell number.

This study also gives us reason to believe that the number of small and of mitral cells in the gray layer of the olfactory bulb is very nearly the same for all individuals with especially close agreement between individuals of the same litter. It seems fairly evident that while external conditions may modify to a considerable extent the size of the brain of the albino rat and especially the size of the olfactory bulbs, the only effect is upon the relative development of the individual cells. The number of cells remains the same. The fibers have yet to be examined.

It is important to bear in mind in a determination of this sort—e.g., the number of mitral cells—that a fixed number, in the physical sense, is not to be expected, for all organisms are normally variable in all of their parts, variability being an essential character for living things; so the number which is obtained gives a mean value which we take to be characteristic for the species under the present conditions, but around which equally characteristic variations also occur.

VI. CONCLUSIONS

1. For bulbs of different ages and sizes, the regions anterior to the cerebrum, which are commonly considered the bulbs, are not strictly homologous, since, in the brains of young or stunted rats, a larger proportion of the bulb lies beneath the cerebrum than in the case of the better developed brains.

2. All layers of the olfactory bulb are about equally concerned in the increase in size or in the arrest of development of the bulb.

3. The small cells of the molecular layer show a larger amount of cytoplasm in large bulbs than in small ones.

4. The number of small cells in the molecular layer, apparently, is not correlated either with age of the rat or size of the bulb. The entire computed number for a small, medium, and large bulb was found to be approximately 1,000,000 cells ± 2 per cent.

5. The mitral cells of small bulbs are smaller, on the average, than those of large bulbs.

6. Within the limits here taken the number of mitral cells is not affected by the age or the size of the bulb.

7. There seems to be some variation between litters in the number of the mitral cells. The average number of mitral cells for 13 bulbs was 76,750, the lowest number being 70,625, and the highest 83,974. The standard deviation σ is 4564 and the probable error of the mean ± 855.

8. When members of the same litter are compared, bulbs stunted by a defective diet or enlarged by exercise show practically the same number of mitral cells as do their controls. The mean difference is −1.8 per cent for the tests.

9. The olfactory bulb size is correlated with cell size and not with cell number.

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ABBREVIATIONS

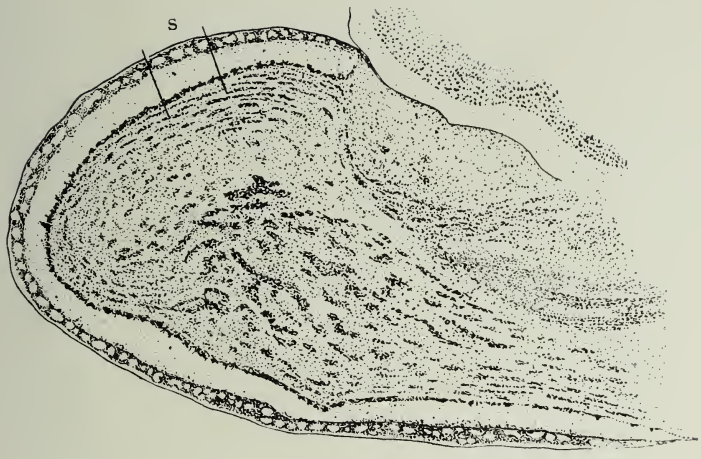
s, areas in figures 1 and 2 enlarged in figures 3 and 4.
fi, outer fiber layer
gl, glomeruli
mo, molecular layer
mi, mitral layer
g, granular layer
f, inner fiber layer
c, cerebrum

PLATE 1

EXPLANATION OF FIGURES

Median longitudinal section through olfactory bulb of F_1, section 3 1/6. F_1, underfed 31 days. Final brain weight, 1.5470 grams, bulb weight, 0.0203 gram. Defective diet. Magnified 24 diameters.

Median longitudinal section through olfactory bulb of F_5, section 5 5/4. F_5, control for F_1. Brain weight, 1.6984 grams, bulb weight, 0.0315 gram. Magnified 24 diameters.



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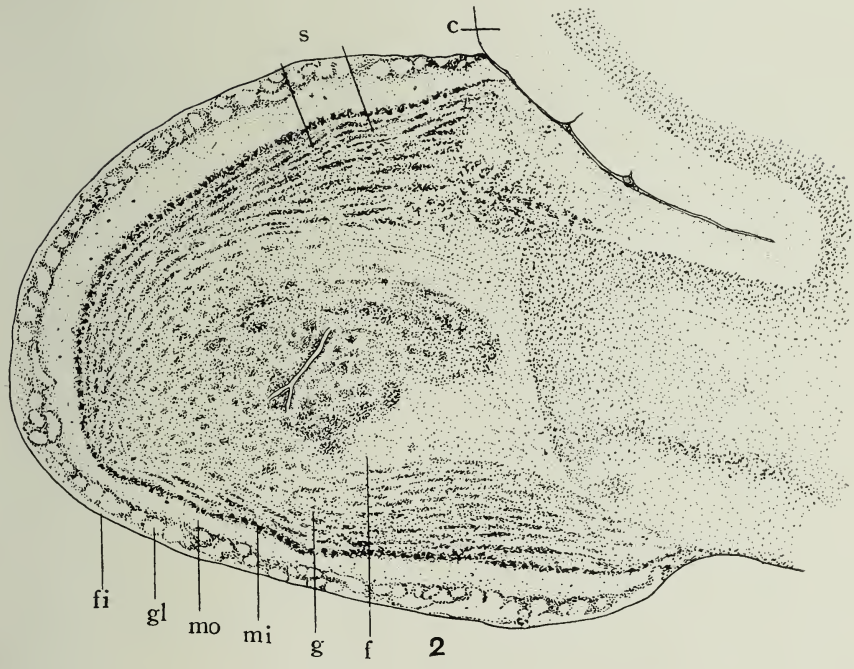

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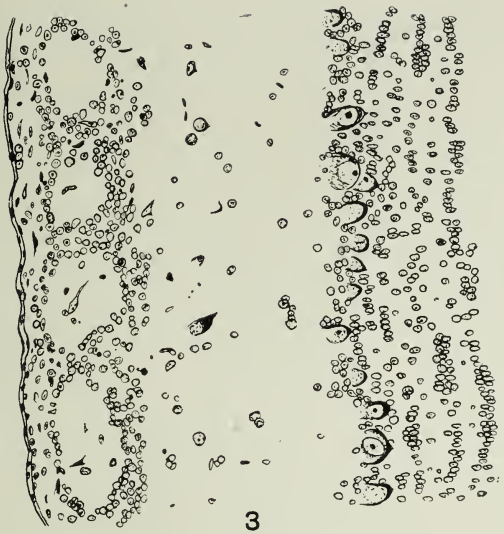
PLATE 2

EXPLANATION OF FIGURES

Portion of section of F_1; area S, in figure 1. Magnified 172 diameters.
Portion of section of F_5; area S, in figure 2. Magnified 172 diameters.



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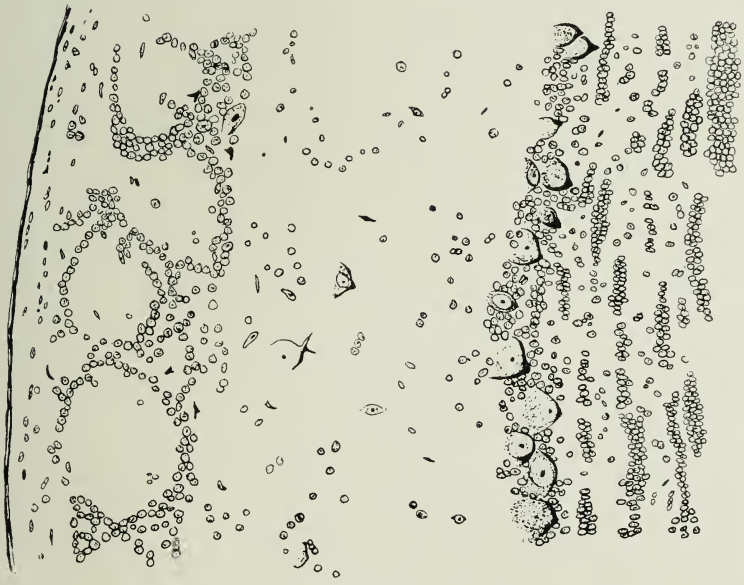

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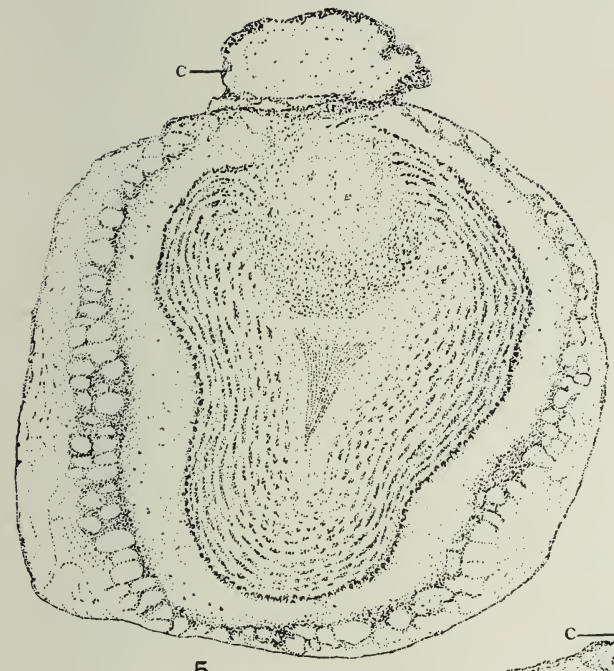
PLATE 3

EXPLANATION OF FIGURES

Cross section of Q_5, section 2 3/3, cut at region where bulb joins cerebrum. Q_5, 30-day control rat, killed when weaned. Brain weight, 1.3164 gram; bulb weight, 0.0238 gram. Magnified 30 diameters.

Cross section of M_4, section 4 1/1, cut same region as figure 5 above. M_4, underfed 30 days. Brain weight, 1.4613 grams; bulb weight, 0.0251 gram. Defective diet. Magnified 30 diameters.



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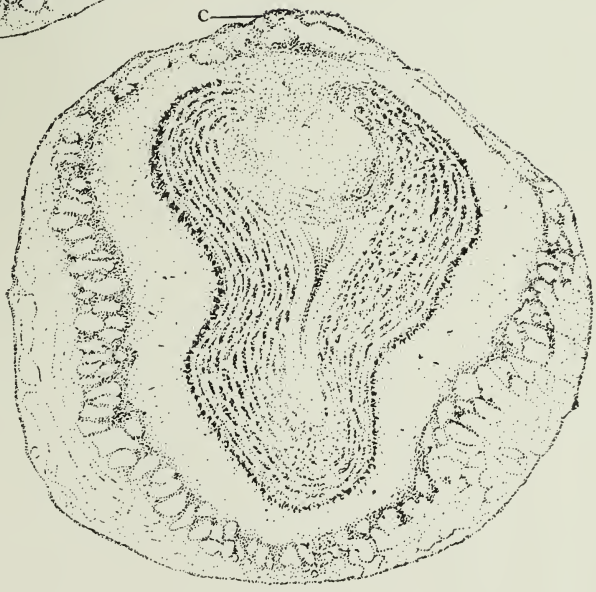

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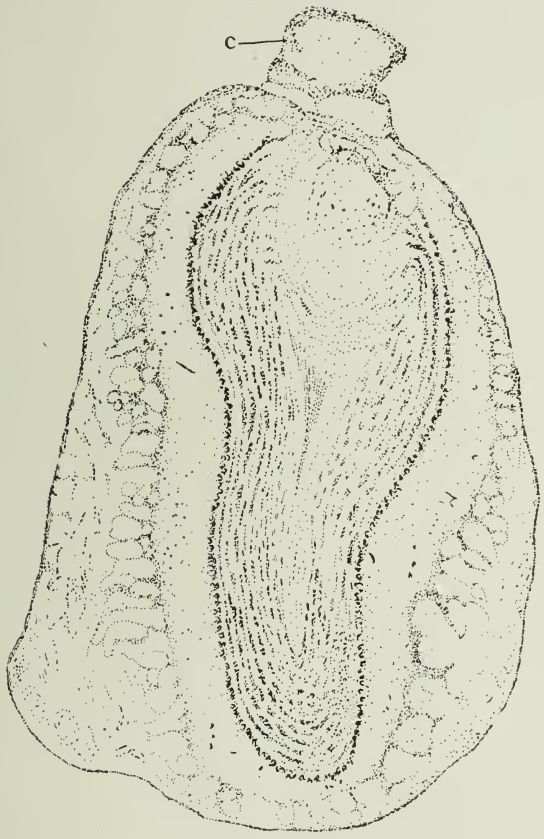
PLATE 4

EXPLANATION OF FIGURES

Cross section of M_5, section 3 6/12, cut same as figure 5 above. M_5, control for M_4. Brain weight, 1.7110 grams; bulb weight, 0.0374 gram. Magnified 30 diameters.



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